IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application Number	: 10/015,939	Confirmation No.:	2933
Applicant	: Prakash KADKADE		
Filed	: December 17, 2001		
Title	: CRYOPRESERVATION OF PLANT CELLS		
TC/Art Unit	: 1651		
Examiner:	: Deborah H. Ware		
Docket No.	: 62698.000010		
Customer No.	: **21967**		

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF MICHAEL E. HORN, PH.D., UNDER 37 C.F.R. 1.132

I, MICHAEL E. HORN, Ph.D., hereby declare as follows:

1. I am a U.S. citizen and currently reside at 52 Princeton Arms North 1, Cranbury, NJ 08512-1104.

2. I am currently employed by Phyton Biotech Inc., which is the assignee of all right, title and interest in the invention disclosed in U.S. Application No. 10/015,939, filed December 17, 2001 (the instant application). I currently hold the title of Director of Cell & Molecular Biology at Phyton Biotech Inc., and have held this title since July 1, 2005.

3. Prior to employment with Phyton Biotech Inc., I was the Director of Cell Biology at Applied Biotechnology Institute, located in College Station, TX 77845, from September, 2004, until June, 2005. I was also the Director of Cell Biology at Prodigene, located in College Station, TX 77845, from June, 1998, until August, 2004.

4. I have received a Bachelor of Arts (B.A.) Degree from the Department of Biology at the University of Missouri, Columbia, MO, in 1977. I have received a Master of Arts (M.A.) Degree from the Department of Biology at the University of Missouri, Columbia, MO, in 1980. I have received a Doctor of Philosophy

(Ph.D.) Degree from the Department of Biology at the University of Illinois, Urbana-Champaign, IL, in 1984.

5. I have been associated with research in the field of plant biotechnology for approximately 21 years, and am an author on approximately 31 peer-reviewed primary publications and review articles, and am a named inventor on 4 issued U.S. patents, as well as 5 pending U.S. patent applications (and the foreign counterparts). A recitation of some of these publications, together with details of my education and my other credentials, are given in my *curriculum vitae* which is attached as Exhibit A.

6. It is my understanding that the U.S. Patent and Trademark Office (USPTO) Examiner in charge of assessing the patentability of the instant Patent Application No. 10/015,939 has rejected the currently pending claims under 35 U.S.C. § 103(a), as allegedly unpatentable over combined references which include Goodrich *et al*, U.S. Patent No. 5,800,978, .

7. I have read, and am familiar with, the following documents:

   a. U.S. Patent Application No. 10/015,939, (the instant application) filed December 17, 2001;

   b. The non-final Office Action mailed August 11, 2005, in the instant U.S. Patent Application No. 10/015,939 (hereinafter the "Office Action");

   c. Goodrich, Jr., *et al*, U.S. Patent No. 5,800,978 (hereinafter "Goodrich"); and

   d. the currently pending claims 1-8, 22, 23, 25, 61-63 and 65-75.

8. According to page 4 of the Office Action, Goodrich is stated to "teach washing the cells after thawing, note column 31, lines 44-46." The referenced citation of Goodrich is to a portion of claim 11 that reads in pertinent part:

   > "thawing said frozen mixture; optionally, reconstituting and/or washing said cells, cell membranes or cell-like materials;"

9. A review of the Goodrich patent specification indicates that the cryopreservation methods of Goodrich are exemplified solely by use of human red blood cells or platelets. For example, in regard to thawing and washing of cells in Example 4, column 12, lines 44-50, Goodrich discloses:

   > The buffer/red cell mixtures were then frozen rapidly in a -80° C ethanol bath, and the frozen samples stored 24 hours at either -80° C or -38° C. Frozen samples were thawed in a 40° C water bath with agitation, and washed in a series of reconstitution buffers

designed to return the cells to isotonic conditions with minimal osmotic shock, and to remove any lysed cell debris.

10. While cryopreservation of animal cultured cells is routine, cryopreservation of cultured plant cells has proven more difficult (*See*, the instant application, page 7, lines 24-26). Based on my experience, a person of ordinary skill in the art of plant cell culture would not view methods exemplified on human blood cells to be *per se* adaptable to plant cells with any reasonable expectation of success. Results from cryopreservation methods of animal cells are just not predicative of results obtained with plant cells.

11. Additionally, and in contrast to the stated objective of the washing in Example 4 of Goodrich, none of the relevant pending claim elements are directed to returning the plant cells to isotonic conditions. The issue of isotonicity highlights the differences between handling animal and plant cells. Because of the plant cell wall, such cells can be turgid in a hypotonic condition or plasmalyzed in a hypertonic situation. Leaving a plant cell in the plasmalyzed condition too long will result in irreparable harm and death. Further, the presence of a relatively thick cell wall also complicates the freezing process considerably. This is because ice crystals can form from water between the plasmalemma and the cell wall such that the crystals will puncture the plasmalemma, resulting in cell death. Thus, it is unreasonable to suppose that any method that was designed for use using red blood cells, which do not have a cell wall, would be useful using plant cells or vice versa.

12. I hereby declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I declare under penalty of perjury that the foregoing is true and correct.

Executed on 9 January 2006

Declarant's Signature: _____

Appendix A

CURRICULUM VITAE OF

MICHAEL E. HORN PH.D.

PROFESSIONAL ADDRESS: Phyton Biotech Inc., 279 Princeton-Hightstown Rd. E. Windsor, NJ 08520-1401; Ph. (609) 426-2528; Fax (609) 426-2521; E-mail: michael.horn@phytonbiotech.com

HOME ADDRESS: 52 Princeton Arms N 1, Cranbury NJ 08512-1104; Ph. (979) 224-0105 (C); E-mail: VonBekX@aol.com

EDUCATION:

Ph.D. 1984 Department of Biology, University of Illinois, Establishment, Optimization and Characterization of Photoautotrophic Soybean Suspension Cultures; Jack M. Widholm, Major Professor

M.A. 1980 Department of Biology, University of Missouri, Characterization of the Electron Transport Chain in Mitochondria from Suspension Cultured Cells of *Nicotiana glutinosa* L.; Dan Mertz, Major Professor

B.A. 1977 Department of Biology, University of Missouri

CAREER SCIENTIFIC ACCOMPLISHMENTS:

- Excellent track record of successful scientific projects covering 14 projects over 25 years.
- Designed world's first useful photosynthetic plant cell culture system, now used world-wide
- One of first examples of genetically engineered Gramineous species, orchardgrass (Patented)
- Established rapid cloning system for several rice varieties, which was adopted by Japanese consortium for that country's rice biotechnology efforts
- Under a contract with a Fortune 200 company, isolated peanut mutants with 1000X the normal level of free proline for food quality enhancement
- Developed novel method for the genetic transformation of maize (Patent Pending)
- Discovered beneficial effects of tobacco MARs (Matrix Attachment Regions) on transformation frequency and foreign gene expression in maize (Patent Pending)
- Coordinated multidisciplinary effort to produce high oleic acid mutants of peanut, now used in several confectionery products and grown on 50% of peanut acreage in the southwest U.S. (three patents)

CAREER BUSINESS/MANAGERIAL ACCOMPLISHMENTS

- 21 years in the plant biotechnology industry
- 16 patent applications, 14 as lead inventor, with four issued. Participated in patent strategy sessions and gave depositions in patent disputes
- Responsible for US-Japan relationship with Kirin Brewery while at Plant Genetics Inc. (PGI), negotiated contract milestones and coordinated reporting

- Wrote proposal for Agrigenetics-Hershey Foods contract; coordinated multidisciplinary research program resulting in the successful commercialization of a new, genetically-modified peanut
- Mycogen representative to North Carolina State MARs Consortium
- 1998-2004 Project Leader for three externally funded projects and several major internal projects. Involved in business development and sales/marketing activities.

PROFESSIONAL ORGANIZATIONS & ACTIVITIES

1982-Present: International Association of Plant Tissue Culture & Biotechnology (IAPTC&B)

1987-Present: Society for In Vitro Biology (SIVB; formerly TCA):

- Editor of "exPlants", Plant Section news column 1990-June 2002
- Secretary of Plant Section 1992-94
- President of Plant Section 1998-2000 and 2000-02 (re-elected)
- Reviewing Editor *In Vitro Cellular & Developmental Biology – Plant* (IVCDB-Plant) 1994-1998
- Associate Editor *In Vitro Cellular & Developmental Biology – Plant* 1998-Present
- Chairman, Publications Committee 2004-2006 and Board Member

2000-Present: *Plant Cell Reports*

- Editor 2000-2005
- Managing Editor 2006-Present

1999-Present: American Association for the Advancement of Science (AAAS)

EMPLOYMENT HISTORY:

July 2005 to Present: ***Director Cell & Molecular Biology,*** **Phyton Biotech Inc.** Responsible for all activity in Cryobiology, Strain Development and TransProtein Production departments. Project leader for establishing protein production platform and developing proprietary new technology surrounding protein production in suspension cultured cells (14 scientists, $1.5M budget)

October 2004 to June 2005: ***Director of Cell Biology,*** **Applied Biotechnology Institute** Responsible for all activity in Cell Biology Department. Project Leader for the HIV Oral Vaccine Project funded by the NIH. This project gained worldwide recognition as a potentially important weapon against HIV and AIDS.

October 2003 to September 2004: ***Director of Cell Biology,*** **ProdiGene** Responsible for all activity in Cell Biology Department and Laboratory Services. Project Leader for the HIV Oral Vaccine Project funded by the NIH. ProdiGene Safety Officer: 1999-2004. Facilities Manager 2002-2004

June 1998 to October 2003: ***Group Leader,*** **ProdiGene**. Group leader for Corn Transformation and Laboratory Services (8 scientists). Responsible for producing corn transformants from all commercial and technology constructs. Also, project leader for three product projects and one major technology project.

February 1991 to May 1998: ***Research Scientist I/II,*** **Agrigenetics/Mycogen**. Project leader for successful novel corn transformation project (supervised 6 scientists). Also, Project Leader for successful nutritional improvement of peanut (supervised 3 scientists).

Personally established ELISA protocols for analysis of transgenic plants. Later, project leader for promoter testing in cotton flower parts; also explored novel methods for cotton transformation. Lead author for six patent applications, three of which have issued.

May 1987 to June 1990: ***Research Scientist*, Plant Genetics, Inc./Calgene, Inc.** Project leader for successful rice tissue culture project (supervised 5 scientists). Project leader for successful peanut mutation project for amino acid overproduction. Responsible for potato transformation project, successfully produced virus-resistant and insect-resistant plants which were field-tested in 1989. At Calgene (5/90-6/90), contributed to efforts at cotton and soybean transformation using particle bombardment of embryogenic suspension cultures. Lead author for five patent applications.

June 1985 to May 1987: ***Post-Doctoral Research Associate*, Ciba-Geigy Biotechnology**. Primary investigator for successful establishment of one of the world's first transformation systems for small grains. Personally performed most of the molecular analyses. Lead author for 2 patent applications, one of which has issued.

April 1984 to June 1985: ***Post-Doctoral Research Associate*, Advanced Genetic Sciences, Inc.** Selection for herbicide-resistant mutants using photosynthetic soybean cell cultures.

June 1980 to April 1984: ***Graduate Research Assistant*, University of Illinois - Champaign-Urbana.** Development of world's first rapidly growing photoautotrophic plant suspension culture. Collaborated on research into somatic hybridization, mutant selection and nitrogen metabolism.

September 1978 to June 1980: ***Graduate Teaching Assistant*, University of Missouri - Columbia**. Taught laboratory sections of General Botany. Performed research on cyanide-resistant mitochondrial respiration in tobacco cell cultures.

PEER REVIEWED PUBLICATIONS:

Kameya, T., **M.E. HORN** and J.M. Widholm. 1981. Hybrid shoot formation from fused *Daucus carota* and *Daucus capillifolius* protoplasts. Z. Pflanzenphysiol. 104:459-466.

HORN, M.E, and D. Mertz. 1982. Cyanide-resistant respiration in suspension cultured cells of Nicotiana glutinosa L. Plant Physiol. 69:1439-1443.

HORN, M.E, J.H. Sherrard and J.M. Widholm. 1983. Photoautotrophic growth of soybean in suspension culture. I. Establishment of photoautotrophic cultures. Plant Physiol. 72:426-429.

HORN, M.E, T. Kameya, J.E. Brotherton and J.M. Widholm. 1983. The use of amino acid resistance and plant regeneration ability to select somatic hybrids between *Nicotiana tabacum* and *N. glutinosa*. Molec. Gen. Genet. 192:235-240.

Nelson, R.S., **M.E. HORN**, J.E. Harper and J.M. Widholm. 1984. Nitrate reductase activity and nitrogenous gas evaluation in heterotrophic, photomixotrophic and photoautotrophic soybean suspension cultures. Plant Sci. Lett. 34:145-152.

Martin, B.A., **M.E. HORN**, J.M. Widholm and R.W. Rinne. 1984. Synthesis, composition and location of glycerolipids in photoautotrophic soybean cell cultures. Biochem. Biophys. Acta 796:146-154.

HORN, M.E, B.V. Conger and C.T. Harms 1988. Plant regeneration from protoplasts of embryogenic suspension cultures of orchardgrass (*Dactylis glomerata* L.). Plant Cell Reports 7:371-374.

HORN, M.E., R.D. Shillito, B.V. Conger and C.T. Harms 1988. Transgenic plants of Orchardgrass (*Dactylis glomerata* L.) from protoplasts. Plant Cell Reports 7:469-472.

HORN, M.E., and J.M. Widholm. 1992. Isolation and characterization of a *Datura innoxia* cell line resistant to ethanol. In Vitro Cell. Dev. Biol. 28P:161-166

HORN, M.E., and J.M. Widholm. 1994. Photoautotrophic growth of soybean cells in suspension culture. III. Characterization of carbon fixation products under high and low CO_2. Plant Cell, Tissue and Organ Cult. 39:239-244

HORN, M.E., and J.M. Widholm. 1994. Photoautotrophic growth of soybean cells in suspension culture. IV. Characterization and time course of free amino acid pools. Plant Cell, Tissue and Organ Cult. 39:245-250

HORN, M.E., 1994. Use of the Skatron Washer in the plant biotechnology industry. Skatron Inks 1:2-4 (feature article)

Streatfield, S.J., J.M. Jilka, E.E. Hood, D.D. Turner, M.R. Bailey, J.M. Mayor, S.L. Woodard, K.K. Beifuss, **M.E. HORN**, D.E. Delaney, I.R. Tizard, and J.A. Howard. 2000. Plant-based vaccines: unique advantages. Vaccine 19:2742-2748

Streatfield, S.J., J.M. Mayor, D.K. Barker, C. Brooks, B.J. Lamphear S.L. Woodard, K.K. Beifuss, D.V. Vicuna, L.A. Massey, **M.E. HORN,** D.E. Delaney, Z.L. Nikolov, E.E. Hood, J.M. Jilka and J.A. Howard. 2002. Development of an edible subunit vaccine in corn against enterotoxigenic strains of *Escherichia coli.* In Vitro Cellular & Developmental Biology – Plant 38:11-17

HORN, M.E., S.L. Woodard, R.C. Clough, S.M. Souers, J.M. Jilka. 2002. Expression of an SIV Protein in Transgenic Maize for Use as an Edible Vaccine and Reagent Supply. Keystone Symposia HIV-1 Protection and Control by Vaccination. Poster (#216) and invited talk.

Hood, E.E.; S.L. Woodard and **M.E. HORN**. 2002. Monoclonal antibody manufacturing in transgenic plants - myths and realities. Current Opinion in Biotechnology 13:630-635

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Bailey, M.R.; S. L. Woodard, E. Callaway, K. Beifuss, M. Magallanes-Lundback, J. R. Lane, **M. E. HORN**, H. Mallubhotla, D. D. Delaney, M. Ward, F. Van Gastel, J. A. Howard, E. E. Hood. 2004. Improved recovery of active recombinant laccase from maize seed. Appl. Microbiol. Biotechnol. 63:390-397

HORN, M.E.; R.L. Harkey, A.K. Vinas, C.F. Drees, D.K. Barker and J.R. Lane. Use of HiII-elite hybrids in *Agrobacterium*-based transformation of maize. In Vitro Cell. Dev. Biol.-Plant (In Review)

Lamphear B.J.; D.K. Barker, C.A. Brooks, D.E. Delaney, J.R. Lane, K. Beifuss, R. Love, K. Thompson, J. Mayor, R. Clough, R. Harkey, M. Poage, C. Drees, **M.E. Horn**, S. J. Streatfield, Z. Nikolov, S. L. Woodard, E. E. Hood, J. M. Jilka, and J. A. Howard. Expression of the Sweet Protein Brazzein in Maize for Production of a New Commercial Sweetener. Plant Biotech. J. (available online)

INVITED REVIEWS, BOOK CHAPTERS

HORN, M.E, and C.C. Dalton. 1984. Photosynthetic cell cultures and their biotechnological applications. Int. Assoc. Plant Tissue Cult. Newsletter (feature article). 43:2-7.

HORN, M.E, and J.M. Widholm. 1984. Aspects of Photosynthetic Plant Tissue Cultures. In: Applications of Genetic Engineering to Crop Improvement. Eds. G.B. Collins and J.F. Petolino. Martinus Nijhoff/Dr. W. Junk, Dordrecht, pp. 113-154.

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Mitten, D.H.; **M.E. HORN**, M. Burrell, and K.S. Blundy. 1990 Tissue Culture Strategies for Potato Transformation and Regeneration. In: Molecular and Cellular Biology of the Potato, Eds, M.E. Vayda and W Park, CAB International, Tucson, AZ USA

HORN, M.E., 1991 Somatic embryogenesis in orchardgrass. In: Plant Tissue Culture Manual: Fundamentals & Applications, Ed. K. Lindsey, Kluwer Academic Publishers, Dordrecht, The Netherlands

HORN, M.E., 1991 Transformation and Regeneration of orchardgrass protoplasts. In: Plant Tissue Culture Manual: Fundamentals & Applications, Ed. K. Lindsey, Kluwer Academic Publishers, Dordrecht, The Netherlands

Hood, E.E.; S.L. Woodard and **M.E. HORN**. 2002. Monoclonal antibody manufacturing in transgenic plants - myths and realities. Current Opinion 13:630-635

Hood, E.E.; **M.E. HORN**, J.A. Howard. 2003. Production and application of proteins from transgenic plants. *In*: I.K. Vasil (ed.) Plant Biotechnology 2002 and Beyond, Kluwer Academic Publ., Netherlands, pp 377-382

Delaney, D.; J. Jilka, D. Barker, P. Irwin, M. Poage, S. Woodard, **M. HORN**, A. Vinas, K. Beifuss, M. Barker, B. Wiggins, C. Drees, R. Harkey, Z. Nikolov, E. Hood, J. Howard. 2003 Production of aprotinin in transgenic maize seeds for the pharmaceutical and cell culture markets. *In*: I.K. Vasil (ed.) Plant Biotechnology 2002 and Beyond, Kluwer Academic Publ., Netherlands, pp 393-394

M.E. HORN, S L Woodard, J.A. Howard. 2004. Plant Molecular Farming: Systems and Products. Plant Cell Reports 22:711-720

ISSUED PATENTS

HORN et al. US Patent #5,596,131 Regeneration of Graminaceous Plants of the Subfamily Pooideae from Protoplasts. Describes the transformation of orchardgrass and wheat protoplasts and regeneration of fertile transformed plants.

HORN et al. US Patent #5,684,232 High Stability Peanut. Describes the mutagenesis of Florunner peanut seed and isolation and partial characterization of a high oleic acid peanut mutant.

HORN et al US Patent #5,948,954 High Stability Peanut. Variety patent covering peanut mutants described in US Patent # 5,684,232

HORN, M.E. and G.E. Hall Jr. European Patent #0970230 An Improved SAR Plant Transformation Process. Describes the use of SARs (MARs) in improving the transformation efficiency and transgene expression level in corn.

HORN et al. US Patent #6,214,405 High Stability Peanut Oil. Describes the oil protected by US Patent #5,684,232.

Five patent applications while at ProdiGene, three as lead inventor. Those published are:

HORN et al. USPApp#20040040061 Expression in plants of HIV-related proteins
HORN USPApp#20030172409 Methods for increasing Biomass of transgenic maize
HORN USPApp#20030172406 Methods for increasing Biomass of transgenic maize

GRANTS

NIH R01 #1R21AI048374-01 "Expression of SIVmac239 gp120 in Transgenic Maize" July 2000 - June 2003; $300,000

NIH SBIR #1R43AI055271-01 "Development of a Plant-based Vaccine against HIV" May 2003 - April 2004; $144,400

REFERENCES

Academic

Dr. Mary Ann Lila, Professor, Natural Resources & Environmental Sciences & Assistant Dean for Research, College of ACES, University of Illinois, 211 Mumford Hall, MC-710, 1301 West Gregory Drive, Urbana, Illinois 61801; Ph. 217-333-0240 or 217-333-5154; Fax 217-333-5816; imagemal@uiuc.edu

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Dr. Jack M. Widholm, Professor, Dept. Agronomy, Plant and Animal Biotechnology Laboratory, 1201. W. Gregory, Urbana, IL 61801 Ph. 217-333-9462, Fax 217-333-4777; widholm@uiuc.edu

Industry

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Dr. M. Keith Redenbaugh, Associate Director, Regulatory Affairs, Licensing and Technology Acquisition, Seminis Vegetable Seeds, 37437 State Highway 16, Woodland, CA 95695 Ph. 530-669-6170, Fax 530-666-4426; Keith.Redenbaugh@seminis.com